

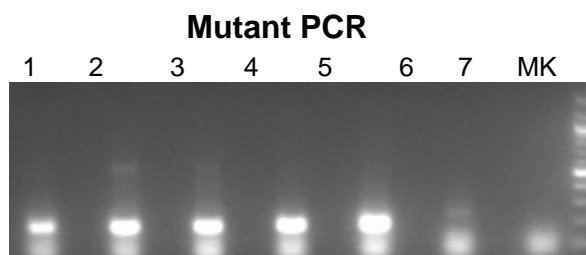
## NIH-0066 Genotyping Strategies

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl <sub>2</sub>	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

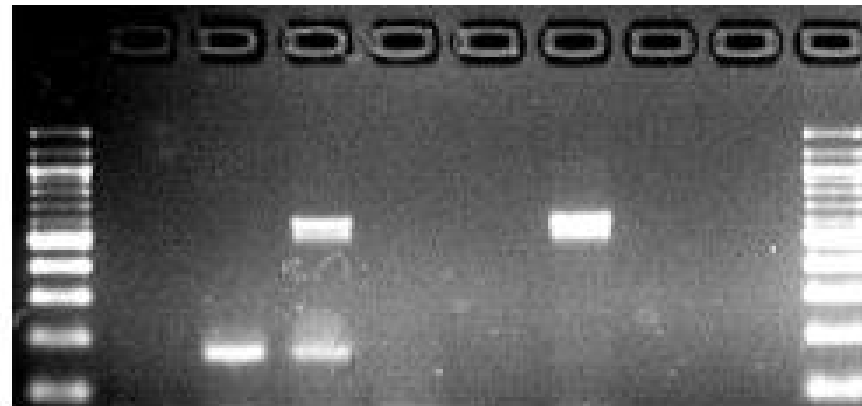
Primer Sequences (5' to 3')	
Mutant PCR: Primer LTR-2 and Primer 0066-3', 201 bp	
Recommended Wt PCR: Primer 0066-5' and Primer 0066-3', 185 bp	
Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC
Primer 0066-3'	TCCCCAACCCACCCCTCTAGTTCA
Primer 0066-5'	GGAATTTTCTGCCTTTTGGGTTAGC

Well	Sample	Genotype
1	87	het
2	99	het
3	106	het
4	122	het
5	<b>ES DNA</b>	het
6	<b>wt lysate</b>	wt
7	<b>water</b>	no amp



# QC Expression

RT	-	+	+	-	+	+	-	-
Actin Primers	-	-	+	-	-	+	-	+



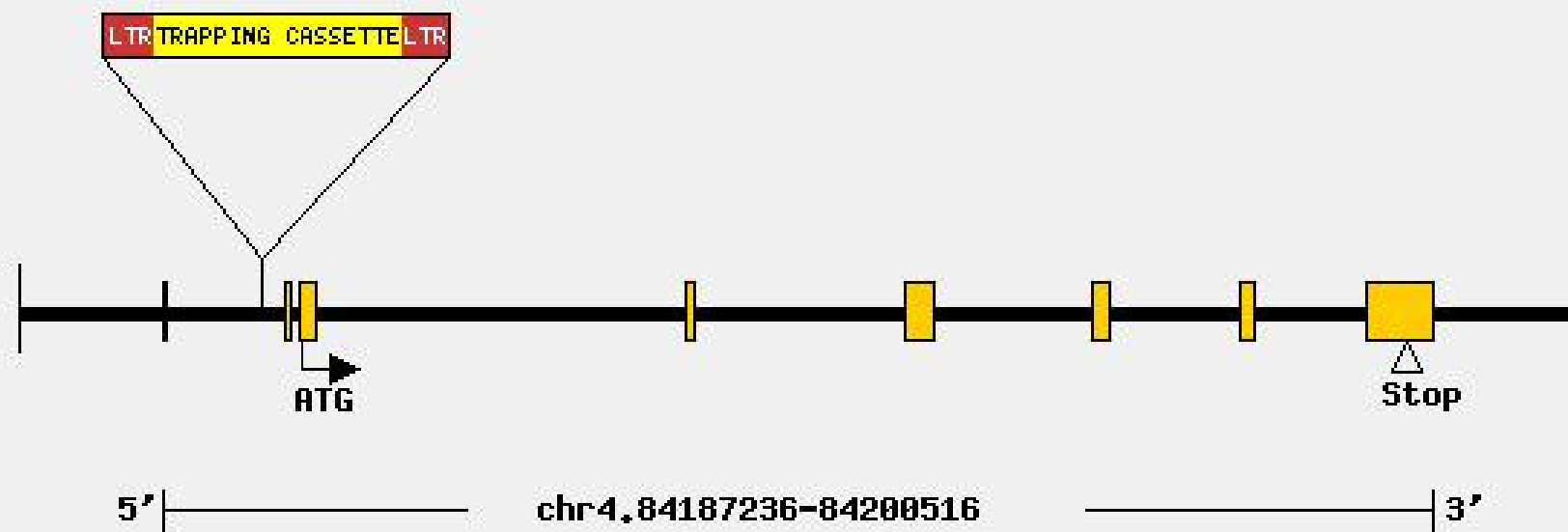
M	—————		—————	—————	M
Genotype	+/+	-/-	No		
Tissue	Brain	Brain	Template		

PCR 35 cycles  
Primers: 1&2

Mouse ID 171

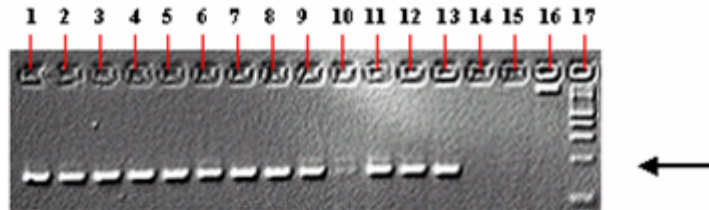
# QC Image

Accession: NM\_007408



# RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2



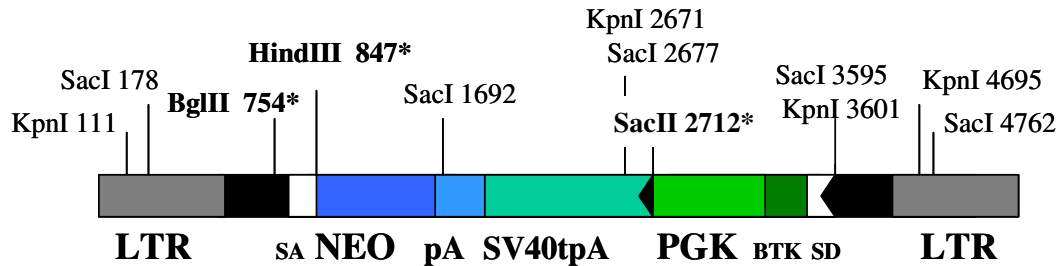
01/10/2004

**Note:** Expected band size denoted by arrow adjacent to 100bp ladder/marker.

## Mouse cDNA Tissues

- 1) Brain
- 2) Spinal Cord
- 3) Eye
- 4) Thymus
- 5) Spleen
- 6) Lung
- 7) Kidney
- 8) Liver
- 9) Skeletal Muscle
- 10) Bone
- 11) Stomach, Small Intestine & Colon
- 12) Heart
- 13) Adipose
- 14) (-) Control
- 15) (+) Control- ES cell cDNA
- 16) (+) Control- Genomic/Lex1 DNA
- 17) 100 bp ladder/marker

## VICTR 48 Omnibank Vector



**Total Size:** 5174 nucleotides

**Non-Cutters:** ApaI, XhoI, XmnI

\* Unique sites

### Location of components in VICTR 48:

LTR (viral long terminal repeat): 1-590, 4585-5174

SA (splice acceptor): 755-847

NEO: 867-1684

pA: 1688-1874

pA (SV40 poly adenylation sequence): 1875-2691

frt sites: 2733-2780, 3613-3661

PGK promoter: 2805-3321

BTK exon: 3356-3580

>VICTR 48

```
TGAAAGACCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
GAACAGCGAGACCACAAGTCGGATGCAACTGCAAGAGGGTTTATTGGATACA
CGGGTACCCGGGCGACTCAGTCAATCGGAGGACTGGCGCGCCGAGTGAGGG
GTTGTGGGCTCTTTTATTGAGCTCGGGGAGCAGAAGCGCGCGAACAGAAGCG
AGAAGCGAACTGATTGGTTAGTTCAAATAAGGCACAGGGTCATTTTCAGGTCC
TTGGGGCACCCCTGGAAACATCTGATGGTTCTCTAGAACTGCTGAGGGCTGG
ACCGCATCTGGGGACCATCTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCT
TACCACAGATATCCTGTTTGGCCCATATTCAGCTGTTCCATCTGTTCTTGGCCC
TGAGCCGGGGCAGGAACTGCTTACCACAGATATCCTGTTTGGCCCATATTCA
GCTGTTCCATCTGTTTCTGACCTTGATCTGAACTTCTCTATTCTCAGTTATGTA
TTTTCCATGCCTTGCAAATGGCGTACTTAAGCTAGCTTGCCAAACCTACA
GGTGGGGTCTTTCATTCCCCCTTTTTCTGGAGACTAAATAAAATCTTTTATTT
TATCTATGGCTCGTACTCTATAGGCTTCAGCTGGTGATATTGTTGAGTCAAAA
CTAGAGCCTGGACCACTGATATCCTGTCTTTAACAAATTGGACTAATCGATAC
CGTCGATCGACCTCGACAGATCTTAAGCCAGTTTTTCGTACCCTTGACTGCGTT
```

TCATCGATTGCTACTAACATTGCCTTTTCCTCCTTCCCTCCCACAGGTGGAA  
GAGCAAGCTTTGATGAGCCGCCACCATGGGATCGGCCATTGAACAAGATGGA  
TTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTTCGGCTATGACTG  
GGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCG  
CAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGA  
ACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCT  
TGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTAT  
TGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGA  
GAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCG  
GCTACCTGCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTA  
CTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCA  
GGGGCTCGCGCCAGCCGAACCTGTTCCGCCAGGCTCAAGGCGCGCATGCCCGAC  
GGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGG  
TGAAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCG  
GATCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTG  
GCGGCGAATGGGCTGACCGCTTCCCTCGTGCTTTACGGTATCGCCGCTCCCGAT  
TCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCA  
ATTCTCTAGAGCTCGGGAGGTAAGTGGAGCGGCCGCAATAAAATATCTTTATT  
TTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGTACTAACATACGC  
TCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCC  
AGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAGGCGGCCCTGCGACT  
CTAGAGGATCTGCGACTCTAGAGGATCATAATCAGCCATAACCACATTTGTAG  
AGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACAT  
AAAATGAATGCAATTGTTGTTGTTAACTTGTTTGTGTTGCAGCTTATAATGGTTA  
CAAATAAAGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACTGC  
ATTCTAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCT  
GCGACTCTAGAGGATCATAATCAGCCATAACCACATTTGTAGAGGTTTTACTTG  
CTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGC  
AATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCA  
ATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGT  
GGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTGCGACTCTAG  
AGGATCATAATCAGCCATAACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAA  
CCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTG  
TTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACA  
AATTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTTGTCCAA  
ACTCATCAATGTATCTTATCATGTCTGGATCCCCGGGTACCGAGCTCGAAGGC  
CGGCCGTTTTAAACCAATCGAATTCCCGCGGCTAGACCCAGCTTTCGGAAGTT  
CCTATTCGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCTCGATATGGTC  
GATCGACCTGCAGGAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAG  
TCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTG  
GCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGGCGCCAACCGGCTCC  
GTTCTTTGGTGGCCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGT  
TCCCCCGCCCCGAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGC  
ACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGGAAGCGG  
GTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGC  
TCAGAGGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAG

GGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTT  
CAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCCTCATCTCCGGGCCTTTC  
GACCTGCAGGCGGCCGCGAATTCAGTACTAGTATTGCAGCGTACGGATCCGCCG  
CCGCCATGGCTCCGGTAGGTCCAGAGTCTTCAGAGATCAAGTCCCACCTTCC  
AAGTCCTGGCATCTCACGACGTCTGGGGAGCTACCTGCATTAAGTCAGAACT  
GAGGTGGGTTTGGGCTGAGGTAGAGCCTGGGCAGAGGCCATAAATTACTTCTT  
GTGGAACCTCTCAAAGGTCGGACAGGAAGCATGGCTGGTTCATATATCTACT  
GCCTCGAATCGATGAATTCGAGCTCGGTACCCGGGGATCGAAGTTCCTATTC  
GGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCTCGACCTGCAGGCATGC  
AAGCTGGGGGGTTCGACGTCGAGAAGGAGTGAGGGCTGGATAAAGGGAGGA  
TCGAGGCGGGGTCGAACGAGGAGGTTCAAGGGGGAGAGACGGGGCGGATGG  
AGGAAGAGGAGGCGGAGGCTTAGGGTGTACAAAGGGCTTGACCCAGGGAGG  
GGGGTCAAAGCCAAGGCTTCCCAGGTCACGATGTAGGGGACCTGGTCTGGG  
TGTCCATGCGGGCCAGGTGAAAAGACCTTGATCTTAACCTGGGTGATGAGGT  
CTCGGTAAAGGTGCCGTCTCGCGGCCATCCGACGTTAAAGGTTGGCCATTCT  
GCAGAGCAGAAGGTAACCCAACGTCTTCTTGGACATCTACCGACTGGTTGT  
GAGCGATCCGCTCGACATCTTCCAGTGACCTAAGGTCAAACCTTAAGGGAGT  
GGTAACAGTCTGGCCCATATTTTCAGACAAATACAGAAACACAGTCAGACAG  
AGACAACACAGAACGATGCTGCAGCAGACAAGACGCGCGGCGCGGCTTCGG  
TCCCAAACCGAAAGCAAAAATTCAGACGGAGGCGGGAACCTGTTTTAGGTTCT  
CGTCTCCTACCAGAACCACATATCCCTCCTCTAAGGGGGGTGCACCAAAGAG  
TCCAAAACGATCGGGATTTTTGGACTCAGGTCCGGGCCACAAAACGGCCCC  
GAAGTCCCTGGGACGTCTCCAGGGTTGCGGCCGGGTGTTCCGAACTCGTCA  
GTTCCACCACGGGTCCGCCAGATACAGAGCTAGTTAGCTAACTAGTACCGAC  
GCAGGCGCATAAAATCAGTCATAGACACTAGACAATCGGACAGACACAGAT  
AAGTTGCTGGCCAGCTTACCTCCCGGTGGTGGGTCCGCTGGTCCCTGGGCAGG  
GGTCTCCCGATCCCGGACGAGCCCCAAATGAAAGACCCCCGCTGACGGGTA  
GTCAATCACTCAGAGGAGACCCTCCCAAGGAACAGCGAGACCACAAGTCGG  
ATGCAACTGCAAGAGGGTTTATTGGATACACGGGTACCCGGGCGACTCAGTC  
AATCGGAGGACTGGCGCGCCGAGTGAGGGGTTGTGGGCTCTTTTATTGAGCT  
CGGGGAGCAGAAGCGCGCGAACAGAAAGCGAGAAGCGAACTGATTGGTTAGT  
TCAAATAAGGCACAGGGTCATTTTCAGGTCCTTGGGGCACCCCTGGAAACATCT  
GATGGTTCTCTAGAAACTGCTGAGGGCTGGACCGCATCTGGGGACCATCTGT  
TCTTGGCCCTGAGCCGGGGCAGGAACTGCTTACCACAGATATCCTGTTTGGCC  
CATATTCAGCTGTTCCATCTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCTT  
ACCACAGATATCCTGTTTGGCCCATATTCAGCTGTTCCATCTGTTCCCTGACCTT  
GATCTGAACTTCTCTATTCTCAGTTATGTATTTTTCCATGCCTTGCAAAATGGC  
GTTACTTAAGCTAGCTTGCCAAACCTACAGGTGGGGTCTTTCA